DETAILED ACTION

Reasons for Allowance

1. An examiner's amendment to the record appears below. Should the changes and/or additions be unacceptable to applicant, an amendment may be filed as provided by 37 CFR 1.312. To ensure consideration of such an amendment, it MUST be submitted no later than the payment of the issue fee.

Authorization for this examiner's amendment was given in a telephone interview with Mr. Daniel Altman (Reg. No. 34,115) on January 29, 2010.

2. The application has been amended as follows:

In the specification:

Replacing paragraph [0001] in page 1 of the specification with ---This application is a continuation-in-part of U.S. application No.10/698,967, filed on October 30, 2003, now abandoned, which is a continuation-in-part of international application No. PCTUS03/12895, which was filed in English on April 24, 2003, and [expected to be] was published in English, and claims the benefit of U.S. Provisional [provisional] Application No. 60/375,472, filed on April 24, 2002.

In the claims:

Cancel claims 80 and 81 and combine claims 89, 90, 220-230, and 234-237 with claims 73, 75, 77-79, 82-88, 91, 92, 215, 217-219, 231-233, and 240.

73. (Currently Amended) A method of determining a definite quantity of a target mRNA encoding a specific sequence in a blood sample comprising:

(a) collecting whole blood;

(b) administering an anticoagulant to the whole blood;

(c) removing erythrocytes and blood components other than leukocytes from the whole blood [to yield] and yielding the leukocytes;

(d) lysing the leukocytes with a lysis buffer containing a known amount of synthesized poly-A spiked control [poly-A] RNA [to produce] and producing a lysate comprising total mRNA containing the target mRNA and said synthesized poly-A spiked control [poly-A] RNA, [thereby obtaining amounts of target mRNA and spiked control RNA respectively,] wherein said synthesized poly-A spiked control RNA is non-homologous to the target mRNA from the whole blood and the amount of said synthesized poly-A spiked control RNA in the lysate does not significantly interfere with quantification of the target mRNA;

(e) transferring the lysate to an oligo(dT)-immobilized plate [to capture] and capturing the total mRNA and said synthesized poly-A spiked control RNA on said oligo(dT)-immobilized plate;

(f) quantifying the target mRNA and the synthesized poly-A spiked control RNA captured on said oligo(dT)-immobilized plate, thereby obtaining [values] the amount of the target mRNA and the amount of the synthesized poly-A spiked control RNA on said oligo(dT)-immobilized plate respectively;

(g) determining the percent recovery of said synthesized poly-A spiked control RNA by dividing the [value] amount of said synthesized poly-A spiked control RNA determined in step (f) by the known amount of said synthesized poly-A spiked control RNA [obtained] in step (d); and

(h) determining the definite quantity of said target mRNA in the blood sample by dividing the [value] amount of said target mRNA determined in step (f) by the percent recovery of said synthesized poly-A spiked control RNA determined in step (g).

75. (Currently Amended) The method of Claim 73, wherein the erythrocytes and blood components are removed by [step (c) comprises] filtration to yield the leukocytes on a filter membrane.

83. (Currently Amended) The method of Claim 73, wherein the erythrocytes and blood components are removed by [step (c) comprises] filtration to yield the leukocytes on a plurality of filter membranes layered together.

88. (Currently Amended) The method of Claim [73] 75, wherein the lysate is produced by lysing said leukocytes on said filter membrane and transferring the [transfer of] lysate to the oligo(dT)-immobilized plate [comprises] by centrifugation.

89. (Currently Amended) The method of Claim [73] 75, wherein the lysate is produced by lysing said leukocytes on said filter membrane and transferring the [transfer of] lysate to the oligo(dT)-immobilized plate [comprises] by vacuum aspiration.

90. (Currently Amended) The method of Claim [73] 75, wherein the lysate is produced by lysing said leukocytes on said filter membrane and transferring the [transfer of] lysate to the oligo(dT)-immobilized plate [comprises] by applying positive pressure.

91. (Currently Amended) The method of Claim 73, wherein the quantification of the target mRNA comprises [cDNA] synthesis of a cDNA from the target mRNA and amplification of the [resulting] cDNA.

92. (Currently Amended) The method of Claim [79] 87, additionally comprising application of specific antisense primers to each well of the multi-well [filter] oligo(dT)-immobilized plate during said lysate transferring step.

215. (Currently amended) A method of high throughput quantification of a target mRNA in a blood sample, comprising the steps of:

(a) collecting whole blood;

(b) administering an anticoagulant to the whole blood;

(c) removing erythrocytes and blood components other than leukocytes from the whole blood by filtration [to yield] and yielding leukocytes on a filter membrane;

(d) lysing the leukocytes on said filter membrane with a lysis buffer comprising antisense primers specific [to] for said target mRNA [to produce] and producing a lysate comprising total mRNA comprising said target mRNA and said antisense primers wherein said target mRNA hybridizes with said antisense primers and forms a complex comprising said target mRNA and said antisense primers [with said antisense primers hybridized thereto];

(e) transferring the lysate to an oligo(dT)-immobilized plate to capture the [total mRNA] complex comprising said target mRNA and said antisense primers on said oligo(dT)-immobilized plate wherein the oligo (dT) is immobilized on the wells of said oligo(dT)-immobilized plate;

(f) removing non-hybridized materials from said oligo(dT)-immobilized plate;

(g) adding a reverse transcriptase to the wells of said oligo(dT)-immobilized plate without addition of further antisense primers, thereby synthesizing cDNAs in a solution of the wells [formed] by extension of both the immobilized oligo(dT) and the antisense primers and forming

an oligo(dT)-derived cDNA and an antisense primer-derived cDNA, wherein the oligo(dT)-derived cDNA [formed by extension of said oligo(dT)] remains immobilized to said plate, and the antisense primer-derived cDNA [formed by extension of the antisense primers] goes into the solution as a result of displacement by the oligo(dT)-derived cDNA [formed by extension of said oligo(dT)] without heat denaturation of said target mRNA and said antisense primer-derived cDNA [formed by extension of the antisense primers]; and

(h) quantifying the target mRNA in the blood sample by amplifying the antisense primer-derived cDNA [in said cDNA] from the solution and quantifying the amplified product produced from the antisense primer-derived cDNA [from said cDNA solution].

217. (Currently amended) The method of Claim 215, [wherein] the lysis buffer further comprises a plurality of different antisense primers specific for different target mRNAs in the blood sample [are present in the lysis buffer].

218. (Currently amended) The method of Claim 217, wherein each of said different mRNAs are quantified using the plurality of different antisense primers specific for the different target mRNAs and the method of claim 215 [by quantifying the cDNA synthesized from the site of hybridization of the antisense primers to said target mRNA].

219. (Currently amended) The method of Claim 217, wherein the antisense primer-derived cDNA [solution] is removed from the plate and the plate with the immobilized oligo(dT)-derived cDNA is stored for future use.

220. (Currently amended) The method of Claim 73, wherein the target mRNA [quantified] is β-actin mRNA.

221. (Currently amended) The method of Claim 73, wherein the target mRNA [quantified] is

CD4 mRNA.

222. (Currently amended) The method of Claim 73, wherein the target mRNA is the mRNA of a translocation gene involved in leukemia [is quantified].

223. (Currently amended) The method of Claim 73, wherein the target mRNA is an [the] mRNA of cancer-specific genes from micrometastatic cancer [is quantified].

224. (Currently amended) The method of Claim 73, wherein the whole blood comprises white blood cells infected by a virus and the target mRNA is a virus-derived mRNA from the infected white blood cells[is quantified].

225. (Currently amended) The method of Claim 224, wherein the virus is HIV and the [quantified] virus-derived mRNA is HIV mRNA.

226. (Currently amended) The method of Claim 225, wherein said quantifying the target mRNA is the quantification of said HIV mRNA and is used to diagnose HIV.

227. (Currently amended) The method of Claim 224, wherein the virus is CMV and the [quantified] virus-derived mRNA is CMV mRNA.

228. (Currently amended) The method of Claim 227, wherein said quantifying the target mRNA is the quantification of said virus-derived mRNA and is used to diagnose CMV.

229. (Currently amended) The method of Claim 224, wherein said quantifying the target mRNA is the quantification of said virus-derived mRNA and is used to monitor blood banks for the presence of a viral disease[s].

230. (Currently amended) The method of Claim 224, wherein said quantifying the target mRNA is the quantification of said virus-derived mRNA and is used to study anti-viral drug sensitivity for said virus.

231. (Currently amended) The method of Claim 73, wherein the target mRNA is an mRNA of apoptosis genes involved in leukemia.

232. (Currently amended) The method of Claim 73, wherein the target mRNA is an mRNA of cytokines.

233. (Currently amended) The method of Claim 73, wherein the target mRNA is an mRNA responsible for apoptosis development.

234. (Currently amended) The method of Claim 73, wherein the target mRNA is an mRNA of DNA-repair genes [is quantified].

235. (Currently amended) The method of Claim 234, wherein said quantifying the target mRNA is the quantification of said mRNA of DNA-repair genes and is used to test the sensitivity of one of DNA-repair genes that encodes said mRNA to radiation.

236. (Currently amended) The method of Claim 73, wherein the target mRNA is an mRNA of allergen response genes [is quantified].

237. (Currently amended) The method of Claim 236, wherein said quantifying the target mRNA is the quantification of said mRNA of allergen response genes and is used to test allergen stimulation.

240. (Currently amended) The method of Claim 217, wherein each of said different mRNAs is [amplified from the cDNAs formed by extension of the immobilized oligo(dT) in step (g)] quantified by amplifying cDNAs produced using the plurality of different antisense primers for the different target mRNAs and the method of claim 215.

3. The following is an examiner's statement of reasons for allowance:

Claims 73, 75-79, 82-92, 215, 217-237 and 240 are allowable in light of applicant's amendments filed on November 30, 2009 and the examiner's amendments. The objection on the specification, the objection on claim 73, and the rejections under 35 U.S.C. 112, first and second paragraphs have been withdrawn in view of applicant's amendments filed on November 30, 2009 and the examiner's amendments. The examiner's amendments are supported by paragraphs [0079], [0081], and [0118] to [0120] of US 2004/0265864 (US publication of this instant case). The closest prior arts in the record are Ishikawa *et al.,* (Clinical Chemistry, 43,764-770, 1997), Mitsuhashi (WO 99/32654, published on July 1, 1999) and Garvin (US 2003/0170669 A1, priority date: April 11, 2000). These prior arts do not teach or suggest the combination of steps d) to h) of claims 73 and 215. These prior arts either alone or in combination with the other art in the record do not teach or reasonably suggest a method of determining a definite quantity of a target mRNA encoding a specific sequence in a blood sample and a method of high throughput quantification of a target mRNA in a blood sample which comprise all limitations recited in claims 73 and 215.

Any comments considered necessary by applicant must be submitted no later than the payment of the issue fee and, to avoid processing delays, should preferably accompany the issue fee. Such submissions should be clearly labeled "Comments on Statement of Reasons for Allowance".

4. Papers related to this application may be submitted to Group 1600 by facsimile transmission. Papers should be faxed to Group 1600 via the PTO Fax Center. The faxing of such papers must conform with the notices published in the Official Gazette, 1096 OG 30

(November 15, 1988), 1156 OG 61 (November 16, 1993), and 1157 OG 94 (December 28, 1993)(See 37 CAR § 1.6(d)). The CM Fax Center number is (571)273-8300.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Frank Lu, Ph.D., whose telephone number is (571)272-0746. The examiner can normally be reached on Monday-Friday from 9 A.M. to 5 P.M.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Dave Nguyen, can be reached on (571)272-0731.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Frank W Lu /
Primary Examiner, Art Unit 1634
February 1, 2010